Journal of Antimicrobial Chemotherapy (2005) **56**, 698–702
doi:10.1093/jac/dki324
Advance Access publication 2 September 2005

JAC

Dissemination of SHV-12 and CTX-M-type extended-spectrum β-lactamases among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* and emergence of GES-3 in Korea

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Received 26 April 2005; returned 5 June 2005; revised 15 August 2005; accepted 15 August 2005

Objectives: **To assess the prevalence and genotypes of Ambler class A extended-spectrum β-lactamases (ESBLs) in Korea.**

Methods: **Clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* collected from 12 Korean hospitals during February–July 2003 were evaluated. Antimicrobial susceptibilities were determined by disc diffusion and agar dilution methods, and the putative ESBL-producing strains were tested by the double-disc synergy method. Detection of genes encoding class A β-lactamases was performed by PCR amplification, and the PCR products were subjected to direct sequencing.**

Results: **The double-disc synergy test showed positive results in 9.3% (23/246) of *E. coli* and 23.0% (55/239) of *K. pneumoniae* isolates. The most prevalent types of Ambler class A ESBLs in *E. coli* isolates were CTX-M-15 (*n* = 4) and CTX-M-3 (*n* = 3), and those in *K. pneumoniae* isolates were SHV-12 (*n* = 30) and CTX-M-3 (*n* = 13). Two isolates produced both SHV-12 and GES-3, simultaneously.**

Conclusions: **CTX-M-type and/or SHV-12 ESBL-producing *E. coli* and *K. pneumoniae* isolates are spreading, and a GES-type ESBL has emerged in Korea.**

Keywords: ESBLs, resistance, prevalence

Introduction

Escherichia coli and *Klebsiella pneumoniae* are the most frequent bacteria that produce SHV- and TEM-type extended-spectrum β-lactamases (ESBLs) and SHV-2a, SHV-12 and TME-52 are common in Korea.[1] CTX-M-type ESBLs, the most widely spread enzymes among non-TEM and non-SHV plasmid-mediated ESBLs, were initially reported in the late 1980s in Europe.[2] At present, the CTX-M family comprises more than 40 enzymes that have similar substrate specificities and inhibitor profiles to TEM and SHV derivatives but show greater hydrolytic activity against cefotaxime than ceftazidime. In Korea, CTX-M-3-, CTX-M-14- and CTX-M-15-producing *E. coli* and *K. pneumoniae* were reported in a survey of 13 Korean hospitals in 2002.[3] GES-type ESBLs are within the Ambler class A but have different substrate specificity. GES-1, which was detected in *K. pneumoniae* in French Guiana, had activity against cefoxitin, whereas GES-2 from *Pseudomonas aeruginosa* in South Africa was active against imipenem.[4] Recently, a few derivatives of GES-type ESBLs have been isolated in Greece and Japan.[5,6]

The aim of the present study was to describe the prevalence and shift of the ESBLs including non-TEM or -SHV ESBLs in *E. coli* and *K. pneumoniae* in recent years in Korea.

Methods

Bacterial strains

Consecutive non-duplicate nosocomial isolates of *E. coli* (*n* = 246) and *K. pneumoniae* (*n* = 239) were collected during February–July

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2003 from 12 hospitals in Korea (Figure 1). *E. coli* J53 Azide^R was used as the recipient strain for conjugation. *E. coli* ATCC 25933 was used as the reference strain for antimicrobial susceptibility testing.

Antimicrobial susceptibility tests and β-lactam resistance transfer assays

Antimicrobial susceptibilities were determined by disc diffusion and agar dilution methods according to the recommendations of the NCCLS.[7,8] ESBL production was detected by the double-disc synergy (DDS) method. Mating experiments were performed as described previously.[1] Transconjugants were selected on MacConkey agar supplemented with ceftazidime (2 mg/L) and sodium azide (150 mg/L; Sigma, St Louis, MO, USA).

Isoelectric focusing

Crude bacterial extracts from clinical isolates were prepared as described previously.[1] Sonic extracts and sample buffer (TEFCO corporation, Tokyo, Japan) were mixed in equal amounts and separated by electrophoresis on precast polyacrylamide gels (pH 3–10, TEFCO corporation, Tokyo, Japan) for 1 h at 100 V, 1 h at 200 V and 40 min at 300 V. β-Lactamase activity was detected with 0.5 mM nitrocefin (Oxoid, Basingstoke, UK).

Molecular analysis

Primers used in this study are listed in Table 1. The templates for PCR amplification in clinical isolates were a whole-cell lysate or a plasmid preparation. The PCR products were then subjected to direct sequencing. Both strands of each PCR product were sequenced twice with an automatic sequencer (model 373A; Applied Biosystems, Weiterstadt, Germany).

Results

In the study period, clinical isolates of *E. coli* (n = 246) and *K. pneumoniae* (n = 239) were obtained from outpatients (43.1 and 23.0%, respectively), inpatients of general wards (46.3 and 60.7%, respectively) and inpatients of intensive care units (ICUs) (10.6 and 16.3%, respectively). They were from urine (52.6%), wound (18.2%), sputum (17.1%), blood (8.2%) and body fluid (3.9%). ESBL production was detected in 9.3% (23/246) of *E. coli* and 23.0% (55/239) of *K. pneumoniae* by the DDS test. DDS-positive isolates were found in all 12 hospitals (Figure 1). The greatest numbers of ESBL-producing isolates were found in a hospital in Suwon city (*E. coli*, 5/20; *K. pneumoniae*, 9/20). The DDS-positive rates were higher in isolates from ICUs (*E. coli*, 23.1%; *K. pneumoniae*, 35.9%) than from outpatients or inpatients of general wards (*E. coli*, 0.9% and 14.0%; *K. pneumoniae*, 0.9% and 24.8%, respectively). Transfer of ceftazidime resistance to the *E. coli* J53 Azide^R recipient by conjugation was successful for only 11 of 23 DDS-positive *E. coli* and 33 of 55 DDS-positive *K. pneumoniae* isolates.

Among 23 and 55 DDS-positive *E. coli* and *K. pneumoniae* isolates, genes encoding TEM-type β-lactamases were detected in 78.3% (18/23) of *E. coli* and 60% (33/55) of *K. pneumoniae*. Most of them were TEM-1 except for one isolate each of *E. coli* and *K. pneumoniae* that had TEM-52. The most common types of class A ESBLs identified were SHV-12 and CTX-M-3 in *K. pneumoniae*, and CTX-M-15 and CTX-M-3 in *E. coli*. GES-3, which was detected for the first time in Korea, was detected in two isolates of *K. pneumoniae* from Bundang city. Six isolates (12.2%) of *K. pneumoniae* and one *E. coli* carried multiple ESBL genes (Table 2). Non-TEM- and non-SHV-type ESBLs including

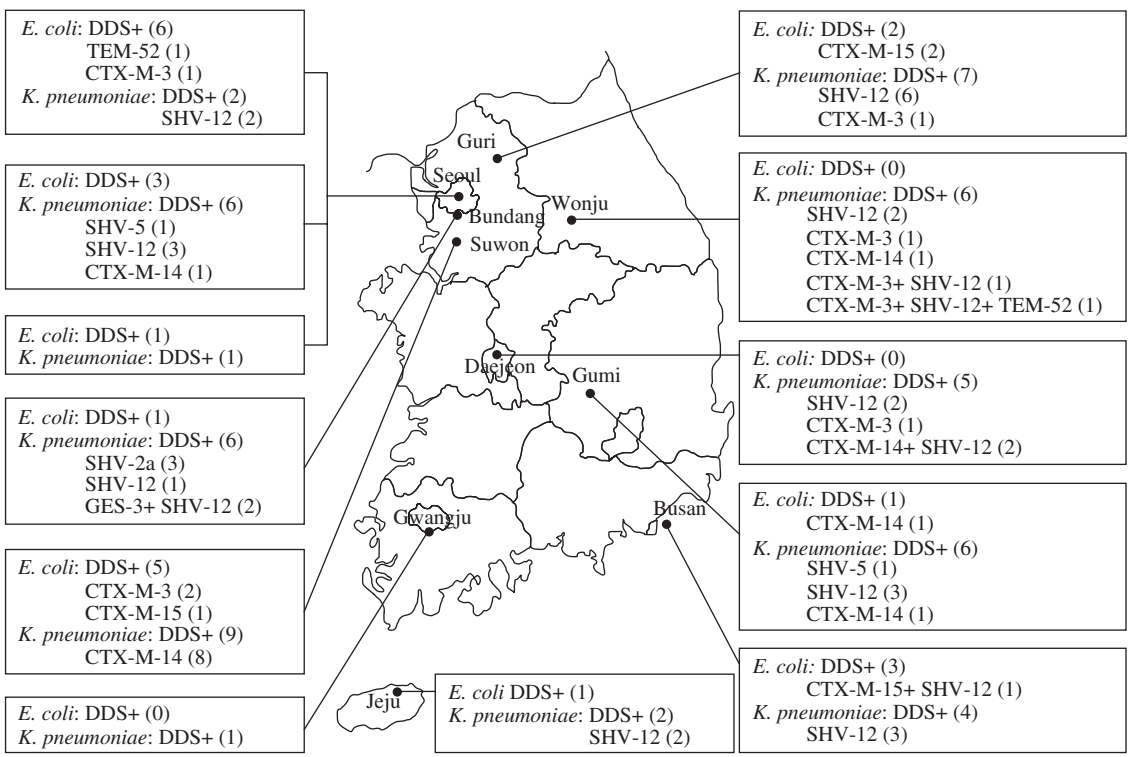

Figure 1. Location of Korean hospitals involved in this survey with the numbers of double-disc synergy-positive (DDS+) isolates and distribution of Ambler class A ESBLs at each hospital in parentheses.

Table 1. Sequences of the primers used to detect Ambler class A β-lactamase genes

Primer name	Oligonucleotide sequence	PCR target	Product size (bp)
TEM-F	5′-ATG AGT ATT CAA CAT TTC CGT-3′	bla_{TEM}	861
TEM-R	5′-TTA CCA ATG CTT AAT CAG TGA-3′		
SHV-F	5′-CCG GGT TAT TCT TAT TTG TCG CT-3′	bla_{SHV}	831
SHV-R	5′-TAG CGT TGC CAG TGC TCG-3′		
C1-F	5′-GGA CGT ACA GCA AAA ACT TGC-3′	bla_{CTX-M} (CTX-M-1 group)	624
C1-R	5′-CGG TTC GCT TTC ACT TTT CTT-3′		
C2-F	5′-CGG TGC TTA AAC AGA GCG AG-3′	bla_{CTX-M} (CTX-M-2 group)	891
C2-R	5′-CCA TGA ATA AGC AGC TGA TTG CCC-3′		
C8-F	5′-ACG CTC AAC ACC GCG ATC-3′	bla_{CTX-M} (CTX-M-8 group)	490
C8-R	5′-CGT GGG TTC TCG GGG ATA A-3′		
C9-F	5′-GAT TGA CCG TAT TGG GAG TTT-3′	bla_{CTX-M} (CTX-M-9 group)	947
C9-R	5′-CGG CTG GGT AAA ATA GGT CA-3′		
PER-1-F	5′-GTT AAT TTG GGC TTA GGG CAG-3′	bla_{PER-1}	855
PER-1-R	5′-CAG CGC AAT CCC CAC TGT-3′		
VEB-F	5′-ACC AGA TAG GAG TAC AGA CAT ATG A-3′	bla_{VEB}	727
VEB-R	5′-TTC ATC ACC GCG ATA AAG CAC-3′		
I/G-F	5′-GTT AGA CGG GCG TAC AAA GAT AAT-3′	bla_{IBC} and bla_{GES}	903
I/G-R	5′-TGT CCG TGC TCA GGA TGA GT-3′		
TLA-F	5′-CGC GAA AAT TCT GAA ATG AC-3′	bla_{TLA}	992
TLA-R	5′-AGG AAA TTG TAC CGA GAC CCT-3′		

PER-1, VEB, IBC and TLA-type ESBLs and members of CTX-M-2 and -8 groups were not detected in this survey. For 14 of 23 (60.9%) DDS-positive *E. coli* and six of 55 (10.9%) DDS-positive *K. pneumoniae* isolates no ESBL was detected. These isolates may have produced another ESBL, which was not determined in this study or might have given positive results for ESBL activity.

All of the 31 isolates producing SHV-12 were resistant to ceftazidime (MIC ≥ 64 mg/L). Five isolates producing both SHV-12 and CTX-M-type ESBLs were highly resistant to both ceftazidime and cefotaxime (both MICs ≥ 256 mg/L). Four isolates producing only CTX-M-14 had more than fourfold higher MICs for cefotaxime than for ceftazidime, but the isolates producing CTX-M-15 had similar levels of MICs for these drugs except one. Two isolates producing both GES-3 and SHV-12 had higher MICs for ceftazidime (≥256 mg/L) than for cefotaxime (64 mg/L). In isoelectric focusing studies, each of SHV-5, SHV-12, TEM-52, CTX-M-3, CTX-M-14, CTX-M-15 and GES-3 enzymes had corresponding pIs at 7.6, 8.2, 6.0, 8.4, 8.1, 8.6, and 6.1, respectively.

Discussion

Compared with a survey in 1997,[9] the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* has increased from 4.8 to 9.3% and 22 to 23%, respectively, in Korea. Past reports showed that the most common ESBL in Korea was TEM-52,[9] but it was detected in only one isolate each of *E. coli* and *K. pneumoniae* in this study. In 1998, about 70% (27/39) of *K. pneumoniae* isolates that produced SHV-type ESBLs were SHV-12, which confers higher levels of resistance against ceftazidime than SHV-2a and SHV-5, and the remaining 30% carried SHV-2a.[10] However, 86% (31/36) of SHV-type ESBLs were SHV-12 and only 8% (n = 3) and 6% (n = 2) were SHV-2a and SHV-5, respectively in this study.

We have previously shown that only 1.7% (9/520) of clinical *E. coli* and *K. pneumoniae* isolates produced CTX-M-type ESBLs in 2002,[3] but the prevalence of these enzymes was increased to 4.4% (26/585) in the present study. CTX-M-3 was the most common enzyme among CTX-M-type ESBLs, and was isolated from five hospitals (Figure 1). CTX-M-15 differs from CTX-M-3 by one amino acid substitution from glycine to aspartate at position 240, and this amino acid change results in increased enzymatic activity against ceftazidime.[2] It was also observed in this study that MICs of ceftazidime for four isolates of *E. coli* that produced CTX-M-15 were all ≥128 mg/L, which was higher than that of isolates producing other CTX-M-type ESBLs. Another noteworthy finding was that five CTX-M-type ESBL-producing isolates also produced SHV-12, simultaneously, and they were highly resistant to both ceftazidime and cefotaxime. Simultaneous production of both cefotaximase and ceftazidimase may confer a higher level of resistance against these oxyimino-cephalosporins in clinical isolates.

GES-3 was originally found in an *E. coli* isolate from a hospital in Greece.[5] GES-type ESBLs have not been reported before in Korea. However, in the present study, we found that two isolates of *K. pneumoniae* from a hospital in Bundang city produced both GES-3 and SHV-12 ESBLs. The MIC of ceftazidime (MIC ≥ 256 mg/L) was more than fourfold higher than that of cefotaxime (MIC 64 mg/L) in both of these isolates, and showed little change when clavulanic acid was added.

The present data suggest that the incidence of isolation of SHV-12 and CTX-M-type ESBLs has increased in *E. coli* and *K. pneumoniae* isolates in Korea. In conclusion, 9.3% of *E. coli* and 23.0% of *K. pneumoniae* isolates from Korea have produced Ambler class A ESBLs. The most common ESBLs in *E. coli* isolates were CTX-M-15 and CTX-M-3, and in *K. pneumoniae* were SHV-12 and CTX-M-3. In addition, a GES-type ESBL has emerged in Korea.

Table 2. Distributions of Ambler class A ESBL genotypes in 78 isolates of *E. coli* and *K. pneumoniae* with MICs of β-lactams

Type of Ambler class A ESBLs	No. of isolate (%)[a]		MIC (mg/L)											
			Ceftazidime			Ceftazidime-clavulanic acid[b]			Cefotaxime			Cefotaxime-clavulanic acid[b]		
	E. coli	*K. pneumoniae*	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
CTX-M-3	3 (1.2%)	11 (4.6%)	16–>256	>256	>256	16–>256	>256	>256	256–>256	>256	>256	32–>256	>256	>256
CTX-M-14	1 (0.4%)	3 (1.3%)	16–64			8–64			256–>256			64–>256		
CTX-M-15	3 (1.2%)		128–>256			64–>256			>256			128–>256		
SHV-2a		3 (1.3%)	16–128			8–64			4–16			0.25–4		
SHV-5		2 (0.8%)	32–256			2–16			4–8			0.25–1		
SHV-12		24 (10.0%)	64–>256	128	>256	2–128	16	64	4–64	16	64	0.25–8	0.5	4
TEM-52	1 (0.4%)		256			32			>256			128		
CTX-M-3+SHV-12		1 (0.4%)	>256			>256			>256			>256		
CTX-M-14+SHV-12		2 (0.8%)	>256			>256			256–>256			256–>256		
CTX-M-15+SHV-12	1 (0.4%)		>256			>256			>256			>256		
GES-3+SHV-12		2 (0.8%)	256–>256			128–>256			64			16–64		
CTX-M-3+SHV-12+TEM-52		1 (0.4%)	>256			>256			>256			>256		
Total	9 (3.7%)	49 (20.5%)												

[a]Fourteen of 23 DDS-positive *E. coli* and 6 of 55 DDS-positive *K. pneumoniae* isolates may have produced another ESBL, which was not determined in this study or might be false positive in ESBL activity.
[b]Clavulanic acid at a fixed concentration of 4 mg/L.

Acknowledgements

This work was supported by the research grant from the Korea Food and Drug Administration (04062HangNaeAn673). We thank J. Lee, J. O. Kang, J. Y. Ahn, J. H. Shin, Y. Uh and W. G. Lee for providing clinical isolates of *E. coli* and *K. pneumoniae*.

Transparency declarations

There are no conflicts of interest.

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